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Environmentally safe molluscicides from two common euphorbiales

Molusquicidas no perjudiciales para el medioambiente obtenidos a partir de dos euforbiáceas

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ABSTRACT

Aqueous extracts of latex and stem bark of *Codiaeum variegatum* and *Croton tiglium* (Euphorbiaceae) have a high molluscicidal activity. It was observed that the molluscicidal activity of extracts of both the plants against two harmful freshwater snails *Lymnaea acuminata* and *Indoplanorbis exustus* was time as well as dose dependent. There was a significant negative correlation between LC50 values and exposure periods thus increase in exposure time, the LC50 of *Croton tiglium* and *Codiaeum variegatum* latices were decreased from 0.060 mg DW/L (24h)> to 0.014 mg DW/L (96h) and 0.381 mg DW/L (24h)> to 0.159 mg DW/L (96h), respectively against *Lymnaea acuminata* and 0.034 mg DW/L (24h)> to 0.009 mg DW/L (96h) and 0.246 mg DW/L (24h)> to 0.030 mg DW/L (96h), respectively against *Indoplanorbis exustus*.

These plant part extracts at higher doses were also lethal to freshwater fish *Channa punctatus*, which shares the habitat with these snails, but the doses LC90, (24h) of snails are safe for fish.

RESUMEN

Los extractos acuosos de latex y corteza del tallo de Codiaeum variegatum y Croton tiglium (Euphorbiaceae) tienen una alta actividad molusquicida. Esta actividad, frente a dos caracoles de agua dulce dañinos, *Lymnaea acuminata* y *Indoplanorbis exustus* , depende tanto del tiempo como de la dosis. Hay una correlación negativa significativa entre los valores LC50 y los periodos de exposición según se aumentan éstos, el LC50 de los latex de *Croton tiglium* y *Codiaeum variegatum* fueron decrecientes 0.060 mg DW/L (24h)> hasta 0.014 mg DW/L (96h) y 0.381 mg DW/L (24h)> hasta 0.159 mg DW/L (96h), respectivamente contra *Lymnaea acuminata* y 0.034 mg DW/L (24h)> hasta 0.009 mg DW/L (96h) y 0.246 mg DW/L (24h)> desde 0.030 mg DW/L (96h), respectivamente contra *Indoplanorbis exustus*.

Estos extractos de partes de plantas a dosis mayores fueron también letales para el pez de agua dulce *Channa punctatus*, que comparte hábitat con estos dos caracoles, pero la dosis LC90, (24h) era inocua para los peces.

KEY WORDS: Molluscicide, *Codiaeum variegatum, Croton tiglium, Lymnaea acuminata, Indoplanorbis exustus*, Euphorbiaceae.

PALABRAS CLAVE: Molusquicida, *Codiaeum variegatum, Croton tiglium, Lymnaea acuminata, Indoplanorbis exustus*, Euphorbiaceae.

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INTRODUCTION

Recent studies have indicated that there are a number of medicinal plants which may be useful for control of snail population and hence control transmission of schistosomiasis and fascioliasis (Medina and Ritchi, 1980; Marston and Hostettman, 1987; Gopalsamy, Gueho, Julien, Owadally and Hostettman, 1990; Singh, Singh and Singh, 1996; Singh and Singh, 1997; Sukumaran, Prashar and Rao, 1994; Marston, Dudan, Gupta, Salis, Correa and Hostettman, 1996; Geerts, Alard, Belot and Sidhom, 1992; Amusan, Msothi and Makhuba, 1997 and Laurens, Fournean, Hoaqnemiller, Care, Bories and Loiseau, 1997).

Molluscicides derived from plants that can be grown in endemic areas of fascioliasis transmission may provide a relatively low cost means for controlling snail intermediate hosts, since expenses of synthesis, marketing and transportation are reduced or eliminated. But, the use of plant products as molluscicides would be justified only, if it can be demonstrated that the effect of the dose needed as molluscicide is non-toxic to other aquatic animals, especially fish.

The present study reports the molluscicidal effect of the two euphorbious plants i.e. *Codiaeum variegatum* and *Croton tiglium* (which is a commonly cultivated as an ornamental plant in gardens, and is usually called a Croton) against the harmful snails *Lymnaea acuminata* and *Indoplanorbis exustus*. These snails are vectors of liver fluke, *Fasciola hepatica* and *Fasciola gigantica*, which causes endemic fascioliasis in Eastern Uttar Pradesh (Singh and Agarwal, 1981). Toxicity experiments have also carried out on freshwater fish *Channa punctatus* (which shares the habitat with snails) for environmental toxicity, if any.

MATERIALS AND METHODS

Latex and stem bark of both the euphorbiales were collected from the Botanical garden of D.D.U. Gorakhpur University, Gorakhpur and identified by Prof. S.K. Singh (taxonomist), Botany Department, D.D.U. Gorakhpur University, Gorakhpur.

Preparation of aqueous extracts of Stem bark and Latex

Stem bark: The fresh stem bark (50mg/5ml) were minced with distilled water homogenized for 5 min and centrifuged at 1000 g for 10 min The supernatant was used as a water extract for the molluscicidal activity.

Latex: The white latex from these plants was drained in glass tubes by cutting their stem apices, this latex was lyophilized at - 40 °C and lyophilized powder was stored for further use. The freeze-dried powder was mixed with appropriate volume of distilled water to obtain the desired concentrations. The wet weight of volume of 1 ml latex of *Codiaeum variegatum* and *Croton tiglium* was 810 mg and 800 mg respectively and dry weight was 305 mg and 300 mg respectively.

Lymnaea acuminata (2.6±0.3 cm in shell height), *Indoplanorbis exustus* (0.87±0.035 cm in shell height) and *Channa punctatus* (10.5±0.9 cm in total length) were collected from Ramgarh Lake of Gorakhpur district, and used as test animals. Toxicity experiments were performed using the method of Singh and Agarwal (1988). Ten experimental animals were kept in glass aquaria, containing 3L of dechlorinated tap water for both the snails. The experimental animals were exposed continuously for 96h to four different concentrations. Control animals were kept under similar conditions without any treatment.

Toxic effect of aqueous extracts of latex and stem bark of both the plants was also studied in mixed populations of fish and snails. In these experiments, a group of 10 snails *Lymnaea acuminata* and 10 fish *Channa punctatus* were put together in 6L dechlorinated tap water. These mixed populations were exposed to previously determined LC90 (24h) of snails for 24h.

Table I. Toxicity (LC10, 50, 90) of aqueous freeze-dried latex extracts of *Croton tiglium* (Family Euphorbiaceae) against *Lymnaea acuminata* at different time intervals. Batches of ten snails were exposed to four different concentrations of aqueous extracts of latex of *Croton tiglium*. Concentrations (Dry weight of latex) given are the final concentrations W/V in aquarium water. Regression coefficient showed that there was significant ($P<0.05$) negative regression between exposure time and different LC values. LCL: lower confidence limit. UCL: upper confidence limit. There was no mortality in control groups.

Tabla I. Toxicidad de extractos acuosos liofilizados de latex (LC10, 50, 90) de Croton tiglium *(Familia Euphorbiaceae) frente a* Lymnaea acuminata *en diferentes intervalos de tiempo. Grupos de 10 caracoles fueron expuestos a 4 diferentes concentraciones de extractos acuosos de latex de* Croton tiglium. *Las concentraciones indicadas (peso seco de latex) son concentraciones finales P/V en el agua del acuario. Los coeficienes de regresión muestran que hay regresiones negativas significativas (P<0.05) entre el tiempo de exposición y diferentes valores de LC. LCL: límite inferior de confianza. UCL: límite superior de confianza. No hubo mortalidad en los grupos de control.*

Exposure periods	Effective dose (W/V) (mg DW/L)	Limit (mg DW/L) LCL	UCL	Slope value	't' ratio	'g' value	Heterogeneity
24h	LC10= 0.015	0.011	0.017				
	LC50=0.06	0.03	0.07	3.98±0.78	5.05	0.15	0.22
	LC90=0.19	0.14	0.42				
48h	LC10= 0.011	0.005	0.013				
	LC50=0.04	0.03	0.06	2.17±0.56	3.86	0.57	0.22
	LC90=0.15	0.07	0.31				
72h	LC10= 0.007	0.004	0.01				
	LC50=0.02	0.017	0.025	2.69±0.50	5.36	0.13	0.77
	LC90=0.061	0.043	0.13				
96h	LC10= 0.04	0.002	0.006				
	LC50=0.014	0.012	0.017	2.38±0.47	4.99	0.15	0.30
	LC90=0.05	0.036	0.106				

Mortality was recorded at 24h intervals up to 96h. Lethal concentrations (LC10, 50, 90) values, Upper and Lower confidence limits (UCL, LCL) and slope values were calculated by the Probit log method using POLO computer programme of Russell et al. (1977). The regression coefficient was determined between exposure time and different values of LC50 (SOKAL AND ROHLF, 1973).

RESULTS

Experimental conditions of water determined by the method of APHA/WPCF (1980). Atmospheric and water temperature was ranging from 30.5 – 31.5 °C and 27.0 – 28.0 °C, respectively. pH of water was 7.3 – 7.5, while dissolved oxygen, free carbon dioxide and bicarbonate alkalinity were ranging from 6.8 – 7.6, 4.4 – 6.5 and 105.0 – 109.0 mg/L, respectively for whole experiments.

(A) Effects on Behavioural changes and Poisoning Symptoms

Exposure to the aqueous extracts of latex and stem bark of *Codiaeum variegatum* and *Croton tiglium* caused significant behavioural changes in the freshwater snails *Lymnaea acuminata* and *Indoplanorbis exustus*. Behavioural changes appear with 5 to 10 min of exposure. The initial 30 – 45 min was a

Table II. Toxicity (LC10, 50, 90) of aqueous freeze-dried latex extracts of *Croton tiglium* (Family Euphorbiaceae) against *Indoplanorbis exustus* at different time intervals. Other details are as given in Table 1.
Tabla II. Toxicidad de extractos acuosos liofilizados de latex (LC10, 50, 90) de Croton tiglium *(Familia Euphorbiaceae) frente a* Indoplanorbis exustus *en diferentes intervalos de tiempo. Resto de detalles como en la Tabla I.*

Exposure periods	Effective dose (W/V) (mg DW/L)	Limit (mg DW/L) LCL	UCL	Slope value	't' ratio	'g' value	Heterogeneity
24h	LC10= 0.009	0.006	0.018				
	LC50=0.034	0.026	0.063	2.36±0.53	4.50	0.18	0.28
	LC90=0.117	0.063	0.549				
48h	LC10= 0.005	0.003	0.008				
	LC50=0.02	0.017	0.025	2.36±0.42	5.56	0.12	0.18
	LC90=0.068	0.044	0.161				
72h	LC10= 0.004	0.002	0.006				
	LC50=0.015	0.012	0.017	2.42±0.40	6.04	0.10	0.26
	LC90=0.050	0.035	0.095				
96h	LC10= 0.03	0.002	0.004				
	LC50=0.009	0.008	0.011	2.96±0.41	7.22	0.48	0.74
	LC90=0.026	0.021	0.037				

Table III. Toxicity (LC10, 50, 90) of aqueous freeze-dried latex extracts of *Codiaeum variegatum* (Family; Euphorbiaceae) against *Lymnaea acuminata* at different time intervals. Other details are as given in Table 1.
Tabla III. Toxicidad de extractos acuosos liofilizados de latex (LC10, 50, 90) de Codiaeum variegatum *(Familia Euphorbiaceae) frente a* Lymnaea acuminata *en diferentes intervalos de tiempo. Resto de detalles como en la Tabla I.*

Exposure periods	Effective dose (W/V) (mg DW/L)	Limit (mg DW/L) LCL	UCL	Slope value	't' ratio	'g' value	Heterogeneity
24h	LC10= 0.159	0.108	0.189				
	LC50=0.381	0.321	0.546	3.41±0.77	4.42	0.19	0.39
	LC90=0.906	0.600	2.589				
48h	LC10= 0.120	0.081	0.147				
	LC50=0.258	0.023	0.291	3.92±0.69	5.62	0.12	0.15
	LC90=0.546	0.432	0.870				
72h	LC10= 0.099	0.066	0.120				
	LC50=0.195	0.171	0.213	4.37±0.69	6.26	0.98	0.20
	LC90=0.381	0.330	0.501				
96h	LC10= 0.090	0.060	0.117				
	LC50=0.159	0.141	0.171	6.60±0.93	7.08	0.07	0.69
	LC90=0.246	0.228	0.279				

Table IV. Toxicity (LC10, 50, 90) of aqueous freeze-dried latex extracts of *Codiaeum variegatum* (Family Euphorbiaceae) against *Indoplanorbis exustus* at different time intervals. Other details are as given in Table 1.
Tabla IV. Toxicidad de extractos acuosos liofilizados de latex (LC10, 50, 90) de Codiaeum variegatum *(Familia Euphorbiaceae) frente a* Indoplanorbis exustus *en diferentes intervalos de tiempo. Resto de detalles como en la Tabla I.*

Exposure periods	Effective dose (W/V) (mg DW/L)	Limit (mg DW/L) LCL	UCL	Slope value	't' ratio	'g' value	Heterogeneity
24h	LC10= 0.045	0.024	0.063				
	LC50=0.246	0.183	0.417	1.78±0.3	5.11	0.1	0.27
	LC90=1.29	0.651	5.793				
48h	LC10= 0.018	0.006	0.027				
	LC50=0.093	0.075	0.117	1.82±0.28	6.42	0.09	0.28
	LC90=0.471	0.318	0.996				
72h	LC10= 0.009	0.003	0.018				
	LC50=0.048	0.036	0.060	1.98±0.28	6.42	0.09	0.92
	LC90=0.222	0.165	0.248				
96h	LC10= 0.006	0.003	0.012				
	LC50=0.030	0.018	0.039	2.16±0.32	6.60	0.08	0.98
	LC90=0.117	0.093	0.168				

period of hyperactivity during which slugish snails moved rapidly in the aquarium water. After some time they started crawling on each other. As the poison enters in the snail's body, a muscular twitching and the snails become spirally twisted, which resulted ataxia, convulsion, paralysis and finally death of snails. Prior to death, there was complete withdrawal of the body inside the shell that indicates nerve poisoning.

(B) Dose-mortality response

LC values (LC10, 50, 90) of aqueous extracts of latex and stem bark of *Croton tiglium* and *Codiaeum variegatum* for period ranging from 24h to 96h for the snails, *Lymnaea acuminata* and *Indoplanorbis exustus* have been given in (Tables I-IV and Figure 1). In case of both the snails toxicity was time as well as dose dependent. There was a significant negative correlation between LC50 values and exposure time (Tables I-IV and Figure 1). Thus increase in exposure time the LC50 of *Croton tiglium* latex decreased from 0.06 mg DW/L (24h);> 0.04 mg DW/L (48h);> 0.02 mg DW/L (72h);> to 0.014 mg DW/L (96h) and 0.034 mg DW/L (24h);> 0.02 mg DW/L (48h); 0.015 mg DW/L (72h);> to 0.009 mg DW/L (96h) in case of *Lymnaea acuminata* and *Indoplanorbis exustus*, respectively (Tables I, II). Same trend of toxicity was observed in case of stem bark extracts of *Croton tiglium* and *Codiaeum variegatum* against both the snails at all the exposure periods (Fig. 1).

Laboratory experiments also indicates that the latex and stem bark extracts of both the plants were more toxic against *Indoplanorbis exustus* than *Lymnaea acuminata* at all the exposure periods.

At higher dose, active moiety of plants, which were effective against the snails, would also cause death amongst the fish. Consequently, a mixed popula-

Table V. Per cent mortality (mean ±SE) of *Lymnaea acuminata* and *Channa punctatus* caused by aqueous extracts of latex and stem bark (i.e. 24h LC90 of snail) of *Codiaeum variegatum* and *Croton tiglium* after 24h exposure period. Each aquarium contained ten fish (*Channa punctatus*) and ten snails (*Lymnaea acuminata*) in 6L dechlorinated tap water. There was no mortality in case of control group.

Table V. Porcentaje de mortalidad (media ±SE) de Lymnaea acuminata *y* Channa punctatus *producida por extractos acuosos de latex y corteza de tallos (i.e. 24h LC90 de caracoles) de* Codiaeum variegatum *y* Croton tiglium *despues de 24 horas de exposición. Cada acuario contenía 10 peces (*Channa punctatus*) y 10 caracoles (*Lymnaea acuminata*) en 6 l de agua de grifo desclorada. No hubo mortalidad en el grupo de control.*

Plants	Plant Parts	Experimental animals	Concentration (mg DW/L) (w/v)	% Mortality
Codiaeum variegatum	Latex	*L. acuminata*	0.906 (LC90)	91.6±2.31
		C. punctatus	-	Zero
	Stem bark	*L. acuminata*	50.14 (LC90)	93.3±1.15
		C. punctatus	-	Zero
Croton tiglium	Latex	*L. acuminata*	0.19 (LC90)	100
		C. punctatus	-	Zero
	Stem bark	*L. acuminata*	35.52 (LC90)	95.0±2.45
		C. punctatus	-	Zero

tions of 10 snails (*Lymnaea acuminata*) and 10 fish (*Channa punctatus*) were treated with the 24h, LC90 of latex and stem bark of *Croton tiglium* and *Codiaeum variegatum*, up to the LC90 doses for snail *Lymnaea acuminata* there was no mortality amongst fish (Table V).

The slope values given in toxicity tables (I – IV) were steep and heterogeneity factor was less than 1.0 indicates the result found to be within the 95% confidence limits of LC values. The regression test ('t' ratio) was greater than 1.96 and the potency estimation test ('g' value) was less than 0.5 at all probability levels.

DISCUSSION

Data of present study shows that the extracts of both the plants caused significant behavioural changes in both the freshwater snails. The most obvious sign of distress in the treated snails were muscular twitching and spiral twisting of the body, followed by crawling on each other. The nature and rapid onset of these behavioural responses indicates that, the latex perhaps contains some neurotoxins, which amongst other think, might be active at the neuromuscular system of the exposed animals. Similar behavioural responses were also observed SINGH AND AGARWAL (1990), in their study on acute toxicity of latices of *Euphorbia royleana*, *Euphorbia antisyphliatica* and *Jatropha gossypifolia* on snail *Lymnaea acuminata*. The behavioural changes are indeed reminiscent to the response of snails to organophosphorus and carbamate pesticides (SINGH AND AGARWAL, 1981).

No such behavioural symptoms and death occurred in control groups indicating that no factor other than plant moieties was responsible for altered behaviour and mortality.

Mortality caused by the plant parts preparation showed a clear significant positive correlation between dose and mortality. For example, for latex of *Croton tiglium* present mortality of snail *Lymnaea acuminata* after 24h was 10% at 0.015 mg DW/L which increased up to 90% at 0.004 mg DW/L (Table I) which

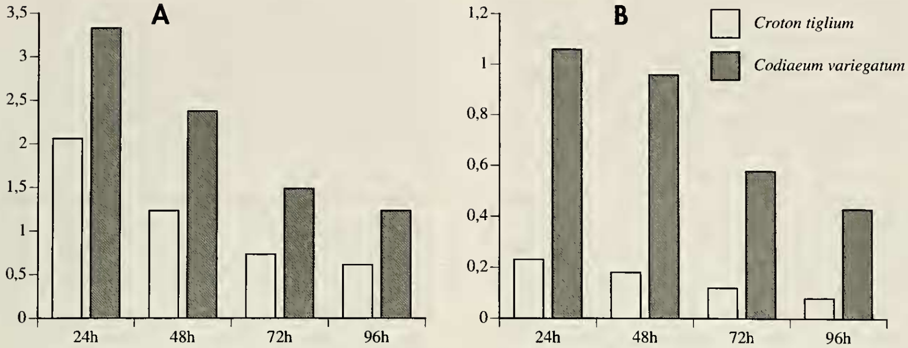

Figure 1. Bar diagram showing the toxicity (LC50; ml/l) of aqueous stem bark extract of *Croton tiglium* and *Codiaeum variegatum* against *Lymnaea acuminata* (A) and *Indoplanorbis exustus* (B) at different time intervals. Batches of 10 snails were exposed to four different dilutions of aqueous extract of bark of *C. tiglium* and *C. variegatum.* Doses are expressed as final concentration (V/V) of stem bark in aquarium. There was no mortality in control group.

Figura 1. Diagrama de barras que muestra la toxicidad (LC50) de extractos acuosos de corteza de tallos de Croton tiglium *y* Codiaeum variegatum *frente a* Lymnaea acuminata *(A) y* Indoplanorbis exustus *(B) a diferentes intervalos de tiempo. Se expusieron grupos de 10 caracoles a 4 concentraciones distintas de extractos de corteza de* C. tiglium *y* C. variegatum. *Las dosis se expresan como concentraciones finales (V/V) de corteza en el acuarion. No hubo mortalidad en el grupo de control.*

in case of *Codiaeum variegatum* mortality it increased from 10% to 90%, when doses increased from 0.159 mg DW/L to 0.090 mg DW/L (Table III). Same trend was also observed in case of stem bark of both the plants at all the exposure periods.

The positive correlation between dose and mortality in all cases was noted because increase concentration of pesticides in aquarium water resulted in more intake or entry of pesticides in the body of animals. This trend is also independent upon several factors such as, rate of penetration, nature of slope, variability and maximal effects of active moieties.

Aqueous preparation of all the plant parts showed a significant negative correlation between LC value and exposure periods e. g LC50 of latex extracts of *Croton tiglium* were decreased from 0.06 mg DW/L (24h); > 0.04 mg DW/L (48h); > 0.02 mg DW/L (72h); > 0.014 mg DW/L (96h) and 0.034 mg DW/L (24h); > 0.02 mg DW/L (48h); > 0.015 mg DW/L (72h); > to 0.009 mg DW/L (96h) in the case of *Lymnaea acuminata* and *Indoplanorbis exustus* respectively (Tables I, II).

Increased in mortality with increased in exposure periods could be affected by several factors, which may be acting separately or conjointly. For example, uptake of active moiety is time dependent, which leads progressive increase the entrance of the drug and its effects in the snail body (SINGH AND AGARWAL, 1988; 1993a; 1993b). Stability (life span) of active moiety of pesticides in environment and the rate of their detoxification in animal body also alter the mortality and exposure periods, relationships (MITRA, SUD AND MITRA, 1978; KOUNDINYA AND RAMAMMURTHY 1979; MATSUMURA, 1985). This possibility cannot be ruled out in case of plant origin pesticides also.

More important is the fact that the latex of these plants is much more toxic than synthetic pesticides. The present study demonstrates that the latex of *Croton tiglium* and *Codiaeum variegatum* have higher molluscicidal activity than any of the prevalent synthetic pyrethroids. Thus, the 24h LC50 of mexacarbamate (3.5 ppm), aldicarb (30.00 ppm), farmothion (27.00 ppm), Cypermethrin (2.5 ppm), permethrin (0.82 ppm) and fenavalerate (2.5 ppm) against the *Lymnaea acuminata* (Singh and Agarwal 1981; Singh and Agarwal 1986; 1987; 1988 and 1991; Sahay, Singh and Agarwal, 1991) is higher than that of the *Croton tiglium* (0.06 ppm) which is about 196 times stronger the standard molluscicides niclosamide (LC50 11.8 ppm) (Singh and Agarwal, 1984).

Statistical analysis of the data on toxicity brings out several important points. The χ^2 test for goodness of fit (Heterogeneity) demonstrated that the mortality counts were not found to be significantly heterogeneous and other variables, e.g. resistance etc. do not significantly affect the LC50 values, as these were found to lie within the 95% confidence limits. The dose mortality graphs exhibit steep slope values. The steepness of the slope line indicates that there is a large increase in the mortality of snails with relatively small increase in the concentration of the toxicant. The slope is, thus an index of the susceptibility of the target animal to the pesticides used. A steep slope is also indicative of rapid absorption and onset of effects. Even though the slope alone is not a very reliable indicator of toxicological mechanism, yet it is a useful parameter (Rand and Petrocelli, 1988) for such a study. Since the LC50 of the latices of different euphorbiales lay within the 95% confidence limits, it is obvious that in replicate test of random samples, the concentration response lines would fall in the same range (Rand and Petrocelli, 1988).

The doses, that can be, used for killing the snails are safe for fish. This is supported by our observations on a mixed population of snails and fish.

In conclusion, it is believed that the extracts of above plants may be used as potent source of molluscicides, because plant products are less expensive, easily available, easily soluble in water and less hazardous to the non-target animals than the synthetic molluscicides.

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